REMARKS

Amendments to the Specification

The title of the application has been replaced by the more descriptive title: "Method of Reducing Hypertension by Administering Flavanol Gylcosides".

Amendments to the Claims

Claim 17 has been amended and new claims 18-24 have been added to recite preferred embodiments of applicants' invention that are more clearly distinguished from the prior art.

Claim 17 has been amended to specify that the method of reducing hypertension comprises administering to the mammal *a foodstuff, dietary supplement or medicament* (page 4, lines 27-31 and page 5, lines 25-25) comprising an effective amount of *a flavonol glucosides* (page 5, lines 21-25).

Claim 18 specifies that the flavanol glucoside recited in claim 17 is a quercetin glucoside (page 5, line 29).

Claim 19 specifies that the flavanol glucoside recited in claim 17 is isoquercitrin (page 5, lines 25-27).

Claim 20 specifies that the flavanol glucoside recited in claim 17 is a naturally occurring flavanol glucoside (page 5, line 31).

Claim 21 specifies that the flavanol glucoside recited in claim 17 is in a substantially isolated and purified form (page 6, lines 1-2).

Claim 22 specifies that the flavanol glucoside recited in claim 17 is combined with a pharmaceutically acceptable carrier or diluent (page 6, line 3).

Claim 23 specifies that the flavanol glucoside recited in claim 17 is at least 90% pure (page 6, line 4).

Claim 24 specifies that the effective amount recited in claim 17 is chosen so as to provide an equivalent of 1 to 20 mg of quercetin aglycon per kg of body weight (BW) of the mammal (page 12, lines 19-20).

Priority

The Examiner asserted that "This application was filed under former 37 CRF §1.60 lacks the information claiming priority to PCT/EP04/06598". Applicants' agent is uncertain how the Examiner concludes that the application was filed under former 37 CRF §1.60 and what information is being referred to.

The attached copies (Exhibit 1) of the original transmittal letter, original declaration (first page) and original Notice of Acceptance demonstrate that the application was filed *under 35 §USC 371 claiming priority under 35 USC §1.19* and fully accepted as such.

Applicants' agent respectfully requests that Examiner to identify what specific further information is required to establish priority.

Claim Rejection – 35 USC §112

Claims 1-13 have been canceled rendering moot their rejection under 35 USC §112.

Claim Rejection – 35 USC §101

Claims 1-13 have been canceled rendering moot their rejection under 35 USC §101.

Claim Rejections – 35 USC § 102

Claim 1-7 and 10-16 were rejected under 35 USC 102(b) as being anticipated by Bovy et al (WO 99/27794 – hereinafter Bovy 99).

Claim 1-5, 8 and 10-16 were rejected under 35 USC 102(a) as being anticipated by Bovy et al (WO 00/04175 – Bovy 00).

Claim 1-5 and 9-16 were rejected under 35 USC 102(a) as being anticipated by Colliver et al (EP 1 254 960 – hereinafter "Collivar").

Claims 1-16 which the Examiner held were directed to a method of use of a plant extract, have been canceled rendering moot their rejection under 35 USC § 102. Furthermore, all pending claims are directed to a methods for reducing hypertension in a mammal, a method which the Examiner has already held is not taught or suggested by Bovy 99, Bovy 00 or Colliver alone or in combination.

Claim Rejections – 35 USC § 103

Claim 17 was rejected under 35 USC 103(a) as being unpatentable over Duarte et al (British Journal of Pharmacology, Vol 133, pages 117-124, 2001 – hereinafter "Duarte" and further in view of Bovy '99, Bovy '00 or Colliver. Applicants respectfully request that the Examiner reconsider this rejection in view of the above amendments and following remarks.

Statement of Facts

Amended Claim 17 is directed to a method for the treatment of hypertension in a mammal. The method comprises administering to the mammal a *foodstuff*, *dietary supplement* or *medicament* comprising an effective amount of a flavonol *glucosides*.

Duarte studied the antihypertensive effects of the flavanoid *quercetin* in spontaneously hypertensive rats (title – emphasis added).

Duarte administered *quercetin* in a vehicle (1ml of 1% methyl cellulose) to rats by *gavage* (feeding tube). Duarte found that *quercetin* administered in this manner induced a significant reduction in systolic, diastolic and mean arterial blood pressure and heart rate in spontaneously hypertensive rats.

Bovy 99, Bovy 00 and Colliver were relied upon for teaching genetically modifying plants such as tomatoes in order to produce increased levels of flavanol glucosides and food extracts from plants.

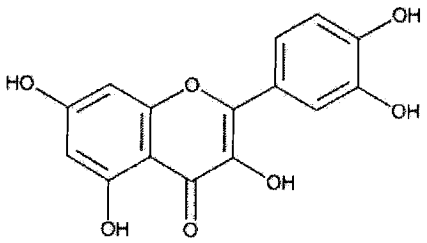
Applicant's arguments

Regarding claim 17

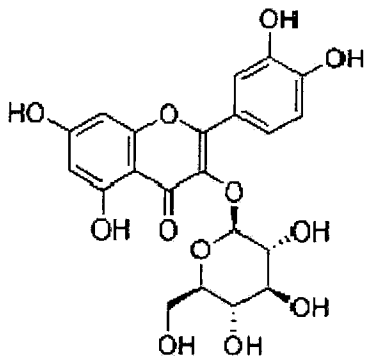
Applicants submit that the combination of references does not render claim 17 obvious under 35 USC 103(a) because the combination does not teach a method for the treatment of hypertension that comprises administering to the mammal a *foodstuff*, *dietary supplement* or *medicament* comprising an effective amount of a *flavonol glucoside.*

Duarte specifically teaches that *quercetin* administered to rats in a vehicle (1ml of 1% methyl cellulose) by *gavage* (feeding tube) reduces hypertension.

Quercetin is a flavanol having the following structure:



In contrast, applicants' claims recite the administration of a flavanol glucoside. A flavanol glucoside includes a glucosyl radical at one or more of the hydroxyl groups of the flavanol. For example, quercetin-3-glucoside (isoquercetrin) has the following chemical structure:



Thus, flavanol glucosides are different chemical entities from flavanols.

Furthermore Durate teaches administration of quercetin (a flavanol) by gavage (feeding tube), while applicants claims are directed to administration by the oral intake of *foodstuff or dietary supplement*. These differences are not trivial.

Applicants' demonstrate in their examples that purified quercetin, the most effective vasodilating material according to Duarte et al (Biochem Pharmacol, 24, 857-862 (1993 - Exhibit 2)), was in fact ineffective in reducing hypertension in the SHR model when delivered in the form of a dietary supplement (**Figures 1-5** comparison curves/bars **BQ** and **TQ**).

This surprising and unexpected finding was recently confirmed by Carlstrom et al (J. Nutr., Vol 137, pages 628-633 (2007 – Exhibit 3)) who also report that *quercetin* when administered as a *dietary supplement,* does <u>not</u> delay the onset or severity of hypertension in spontaneously hypertensive rats. In contrast, Carlstrom found that administration of the quercetin by gavage did reduce hypertension in agreement with

the findings of Duarte. Carlstrom concludes that the data suggests that the mode of delivery is a *critical determinant* in whether quercetin provides cardiovascular benefits.

Thus, the primary reference Durate neither teaches nor suggests a method to reduce hypertension by administering a *flavanol glucoside* in a *foodstuff or dietary supplement*.

Neither Bovy 99, Bovy 00 nor Colliver remedy the shortcomings of Duarte as a prior art reference. The Examiner pointed out that all three references disclose that flavanoids have been reported to exhibit a vasodilatory effect. However, all three references site a common references, Cook et al, Nutritional Biochemistry, 7, 66-76 (1996) for support (copy attached). Cook et al on pages 66 and 73 in turn ultimately cites Duarte et al, Biochem Pharmacol, 24, 857-862 (1993 – Exhibit 4) for their support (Ref 6 – Exhibit 1). The Duarte references deals only with flavanols and flavones and specifically states that quercetin is the most potent of all the compounds tested. The Duarte reference is silent regarding flavanol glucosides.

Applicants respectfully submit that absent a teaching or suggestion of a method for the treatment of hypertension in a mammal that includes administering a foodstuff, dietary supplement or medicament comprising a flavonol glucosides, the combination of references does not present a prima facie case of obviousness over claim 17.

Applicants further submit that new claims 18-24 are also non-obvious over the combination of Duarte and Bovy 99, Bovy 00 or Colliver at least because these claims incorporate all the limitations recited in claim 17 which are neither taught or suggested by the combination of references.

In view of the above amendments and remarks, applicants respectfully request that the 103(a) rejection be reconsidered and withdrawn and that all pending claims be allowed.

If a telephone conversation would be of assistance in advancing prosecution of the applicant, applicant's undersigned agent invites the Examiner to telephone him at the number provided below.

Respectfully submitted,

/ Michael P. Aronson /

Michael P. Aronson
Registration No. 50,372
Agent for Applicant(s)

MPA/sm
(201) 894-2412

EXHIBIT 1

Copies of original transmittal letter, original Declaration and Notice of Acceptance

Express Mail #EJ 622 653 062 US

FORM PTO-1390 U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE (REV. 10-95) TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. § 371		ATTORNEY'S DOCKET NUMBER T3103(C) U.S. APPLICATION NO. (If known, see 37 CFR ɔ 1.5)
INTERNATIONAL APPLICATION NO. PCT/EP2004/006598	INTERNATIONAL FILING DATE 18 JUNE 2004	PRIORITY DATE CLAIMED 31 JULY 2003

TITLE OF INVENTION
USE OF PLANTS WITH INCREASED LEVELS OF FLAVONOL GLYCOSIDES IN REDUCING HYPERTENSION

APPLICANT(S) FOR DO/EO/US

VERHOEYEN, MARTINE ELISA

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a submission under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a submission under 35 U.S.C. 371.
3. ☐ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (21) indicated below.
4. ☒ The US has been elected (Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. §371(c)(2))
   a. ☐ is attached hereto (required only if not communicated by the International Bureau).
   b. ☒ has been communicated by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
   a. ☐ is attached hereto.
   b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. §371(c)(3))
   a. ☐ are transmitted herewith (required only if not communicated by the International Bureau).
   b. ☐ have been communicated by the International Bureau.
   c. ☐ have not been made, however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. §371(c)(3)).
9. ☒ An oath or unexecuted declaration of the inventor(s) (35 U.S.C. §371(c)(4)).
10. ☐ An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. §371(c)(5)).

Items 11. To 16. Below concern document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 C.F.R. §§ 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 C.F.R. §§3.28 and 3.31 is included.
13. ☒ A preliminary amendment.
14. ☐ An Application Data Sheet under 37 CFR § 1.76.
15. ☐ A substitute specification.
16. ☐ A power of attorney and/or change of address letter.
17. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13*ter*.2 and 37 CFR 1.821- 1.825.
18. ☐ A second copy of the published International Application under 35 U.S.C. 154(d)(4).
19. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
20. ☐ Other items or information:

EJ622653062US

COMBINED DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY (includes Reference to PCT International Applications)	ATTORNEY'S DOCKET NUMBER T3103(C)

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

USE OF PLANTS WITH INCREASED LEVELS OF FLAVONOL GLYCOSIDES IN REDUCING HYPERTENSION

the specification of which (check only one item below):

☐ is attached hereto.

☐ was filed as United States application Serial No on and was amended on (if applicable)

☒ was filed as PCT International application PCT/EP2004/006598 on 18 Jun 2004 and was amended under PCT Article 19 on (if applicable)

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the patentability of this application in accordance with Title 37, Code of Federal Regulations, §1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code §119 of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed:

PRIOR FOREIGN/PCT APPLICATION(S) AND ANY PRIORITY CLAIMS UNDER 35 U.S.C. 119:

COUNTRY (if PCT indicate PCT)	APPLICATION NUMBER	DATE OF FILING (day,month,year)	PRIORITY CLAIMED UNDER 35 USC 119
United Kingdom	**0317985.0**	**31 July 2003 (31.07.03)**	☒ **YES** ☐ **NO**

I hereby claim the benefit under Title 35, United States Code §120 of any United States application(s) or PCT international application(s) designating the United States of America that is/are listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in that /those prior application(s) in the manner provided by the first paragraph of Title 35, United States Code §112. I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations §1.56 (a) which occurred between the filing date of the prior application(s) and the national or PCT international filing date of this application.

PRIOR U.S. APPLICATIONS OR PCT INTERNATIONAL APPLICATIONS DESIGNATING THE U.S. FOR BENEFIT UNDER 35 U.S.C. 120.

U.S. APPLICATION(S)		**STATUS (CHECK ONE)**		
U.S. APPLICATION NUMBER	U.S Filing Date	PATENTED	PENDING	ABANDONED
PCT APPLICATIONS DESIGNATING THE U.S.				
PCT APPLICATION NUMBER	PCT Filing Date	U.S Serial Numbers Assigned (if any)		
PCT/EP2004/006598	18 Jun 2004			

UNITED STATES PATENT AND TRADEMARK OFFICE

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United States Patent and Trademark Office
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U.S. APPLICATION NUMBER NO.	FIRST NAMED APPLICANT	ATTY. DOCKET NO.
10/566,806	Martine Elisa Verhoeyen	T3103C

201
UNILEVER INTELLECTUAL PROPERTY GROUP
700 SYLVAN AVENUE,
BLDG C2 SOUTH
ENGLEWOOD CLIFFS, NJ 07632-3100

E.P

INTERNATIONAL APPLICATION NO.	
PCT/EP04/06598	
I.A. FILING DATE	PRIORITY DATE
06/18/2004	07/31/2003

CONFIRMATION NO. 8052
371 ACCEPTANCE LETTER

OC000000027998110

Date Mailed: 01/31/2008

NOTICE OF ACCEPTANCE OF APPLICATION UNDER 35 U.S.C 371 AND 37 CFR 1.495

The applicant is hereby advised that the United States Patent and Trademark Office in its capacity as a Designated / Elected Office (37 CFR 1.495), has determined that the above identified international application has met the requirements of 35 U.S.C. 371, and is ACCEPTED for national patentability examination in the United States Patent and Trademark Office.

The United States Application Number assigned to the application is shown above and the relevant dates are:

12/17/2007	12/17/2007
DATE OF RECEIPT OF 35 U.S.C. 371(c)(1), (c)(2) and (c)(4) REQUIREMENTS	DATE OF COMPLETION OF ALL 35 U.S.C. 371 REQUIREMENTS

A Filing Receipt (PTO-103X) will be issued for the present application in due course. **THE DATE APPEARING ON THE FILING RECEIPT AS THE " FILING DATE" IS THE DATE ON WHICH THE LAST OF THE 35 U.S.C. 371 (c)(1), (c)(2) and (c)(4) REQUIREMENTS HAS BEEN RECEIVED IN THE OFFICE. THIS DATE IS SHOWN ABOVE.** *The filing date of the above identified application is the international filing date of the international application (Article 11(3) and 35 U.S.C. 363).* Once the Filing Receipt has been received, send all correspondence to the Group Art Unit designated thereon.

The following items have been received:

- Copy of the International Application filed on 01/30/2006
- Copy of the International Search Report filed on 01/30/2006
- Preliminary Amendments filed on 01/30/2006
- Information Disclosure Statements filed on 12/17/2007
- Oath or Declaration filed on 12/17/2007
- U.S. Basic National Fees filed on 01/30/2006
- Priority Documents filed on 01/30/2006
- Specification filed on 01/30/2006
- Claims filed on 01/30/2006
- Abstracts filed on 01/30/2006
- Drawings filed on 01/30/2006

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Received
Unilever Patent Dept.
Englewood Cliffs, NJ

FORM PCT/DO/EO/903 (371 Acceptance Notice)

EXHIBIT 2

Duaret et al. Biochem Pharmacol, 24 857-862 (1963)

General Pharmacology: The Vascular System
Volume 24, Issue 4, July 1993, Pages 857-862

doi:10.1016/0306-3623(93)90159-U | How to Cite or Link Using DOI

General paper

Vasodilatory effects of flavonoids in rat aortic smooth muscle. Structure-activity relationships

Juan Duarte[1], Francisco Pérez Vizcaíno[2], Pilar Utrilla[1], José Jiménez[1], Juan Tamargo[2] and Antonio Zarzuelo[1]

[1]Department of Pharmacology, School of Pharmacy, Universidad de Granada, 18071 Granada, Spain [Tel. 34-58-243889; Fax 34-58-243893]

[2]Department of Pharmacology, School of

Medicine, Universidad Computense de Madrid, 28040 Madrid, Spain

Received 25 January 1993. Available online 8 November 2002.

Abstract

1. 1. Flavonoids relaxed the contractions induced by noradrenaline, KCl or phorbol 12-myristate, 13-acetate in rat aortic strips, the order of potency being: flavonols (quercetin, kaempferol, pentamethylquercetin) > flavones(luteolin, apigenin) > flavanols((+)-catechin, (−)-epicatechin) which correlates with the reported order of potency to inhibit protein kinase C.

2. 2. The relaxant effects of kaempferol and luteolin were slightly potentiated by isoprenaline and those of pentamethylquercetin, kaempferol and apigenin by sodium nitroprusside.

3. 3. It is concluded that the main vasodilatory mechanism of flavonoids seems to be the inhibition of protein kinase C. Inhibition of cyclic nucleotide phosphodiesterases or decreased Ca^{2+} uptake may also contribute to their vasodilatory effects.

To whom all correspondence should be addressed.

General Pharmacology: The Vascular System
Volume 24, Issue 4, July 1993, Pages 857-862

EXHIBIT 3

Carlstrom et al. J. Nutr., 137, 628-633 (2007)

 J. Nutr. 137:628–633, March 2007

Nutrition and Disease

A Quercetin Supplemented Diet Does Not Prevent Cardiovascular Complications in Spontaneously Hypertensive Rats[1]

Justin Carlstrom[2,5], J. David Symons[2,5,*], Tzu Ching Wu[2], Richard S. Bruno[4], Sheldon E. Litwin[3] and Thunder Jalili[2,6,*]

[2] College of Health, [3] Division of Cardiology, University of Utah, Salt Lake City, UT 84112 and [4] Department of Nutritional Sciences, University of Connecticut, Storrs, CT 06269

[*] To whom correspondence should be addressed. E-mail: thunder.jalili@utah.edu or 00295675.acs.unc@hsc.utah.edu .

Diets high in quercetin may decrease the risk of developing cardiovascular disease. We tested whether quercetin delays or reduces the severity of hypertension, vascular dysfunction, or cardiac hypertrophy in the spontaneously hypertensive rat (SHR). Normotensive, 5-wk-old SHR consumed standard (n = 18) or quercetin-supplemented diet (1.5 g quercetin/kg diet, n = 22, SHR-Q) for 5 or 11 wk. Wistar Kyoto rats (WKY, n = 19), fed a standard diet, served as controls. At 16 wk, plasma quercetin, measured by HPLC, was 2.09 ± 0.33 μmol/L in SHR-Q and below assay detection limits in SHR and WKY rats. At 10 and 16 wk of age, arterial blood pressure and heart weight:body weight were not different between SHR and SHR-Q. At 16 wk, cardiac function (echocardiography), vascular morphology (hematoxylin and eosin staining of aortae), and resistance and conductance vessel reactivity (wire myography) was unchanged in SHR vs. SHR-Q. Thus, a quercetin-supplemented diet does not delay the onset or lessen the severity of cardiovascular complications that develop in SHR. These findings contrast with previous reports of cardiovascular protection when quercetin was delivered via oral gavage. To determine whether the efficacy of quercetin depends on its method of delivery, 15-wk-old SHR were

given quercetin (10 mg/kg) once daily via oral gavage for 4 consecutive days. Arterial blood pressure (mm Hg) was lower in gavaged SHR (148 ± 5) than in SHR-Q (162 ± 2, $P < 0.02$) and SHR (168 ± 3, $P < 0.001$). These data suggest that mode of delivery is a critical determinant in whether quercetin provides cardiovascular benefits.

This article has been cited by other articles:

EXHIBIT 4

Nutritional Biochemistry
7, 66-76 (1996)

ELSEVIER

Review

Flavonoids—Chemistry, metabolism, cardioprotective effects, and dietary sources

N.C. Cook and S. Samman

Human Nutrition Unit, Department of Biochemistry, University of Sydney, Sydney, Australia

Flavonoids are a group of polyphenolic compounds, diverse in chemical structure and characteristics, found ubiquitously in plants. Therefore, flavonoids are part of the human diet. Over 4,000 different flavonoids have been identified within the major flavonoid classes which include flavonols, flavones, flavanones, catechins, anthocyanidins, isoflavones, dihydroflavonols, and chalcones. Flavonoids are absorbed from the gastrointestinal tracts of humans and animals and are excreted either unchanged or as flavonoid metabolites in the urine and feces. Flavonoids are potent antioxidants, free radical scavengers, and metal chelators and inhibit lipid peroxidation. The structural requirements for the antioxidant and free radical scavenging functions of flavonoids include a hydroxyl group in carbon position three, a double bond between carbon positions two and three, a carbonyl group in carbon position four, and polyhydroxylation of the A and B aromatic rings. Epidemiological studies show an inverse correlation between dietary flavonoid intake and mortality from coronary heart disease (CHD) which is explained in part by the inhibition of low density lipoprotein oxidation and reduced platelet aggregability. Dietary intake of flavonoids range between 23 mg/day estimated in The Netherlands and 170 mg/day estimated in the USA. Major dietary sources of flavonoids determined from studies and analyses conducted in The Netherlands include tea, onions, apples, and red wine. More research is needed for further elucidation of the mechanisms of flavonoid absorption, metabolism, biochemical action, and association with CHD. (J. Nutr. Biochem. 7:66–76, 1996.)

Keywords: flavonoids; chemical structure; metabolism; low density lipoprotein; oxidation; platelet aggregation; coronary heart disease; diet

Introduction

Flavonoids are a group of polyphenolic compounds diverse in chemical structure and characteristics. They occur naturally in fruit, vegetables, nuts, seeds, flowers, and bark and are an integral part of the human diet.[1–3] They have been reported to exhibit a wide range of biological effects, including antibacterial, antiviral,[4] anti-inflammatory, antiallergic,[1,4,5] and vasodilatory[6] actions. In addition, flavonoids inhibit lipid peroxidation (LPO)[2,7] platelet aggregation,[8–12,15] capillary permeability, and fragility,[13,14] and the activity of enzyme systems including cyclo-oxygenase and lipoxygenase.[1,5,15,16] Flavonoids exert these effects as antioxidants, free radical scavengers,[4,17–19] and chelators of divalent cations.[20]

Less is known about the absorption and metabolism of flavonoids, at the usual levels of dietary intake. They are believed to be nontoxic[1] and if absorbed and biologically active in vivo may prevent free radical mediated cytotoxicity and LPO,[21] which is associated with cell aging and chronic diseases such as atherosclerosis.[22]

Much evidence suggests that peroxidation of low density lipoproteins (LDL) is positively associated with atherogenesis.[23–25] Frankel et al.[26] reported that phenolic compounds (including flavonoids and nonflavonoid polyphenols) isolated from red wine inhibit copper catalyzed oxidation of LDL in vitro. It is postulated that the antioxidant and free

Address reprint requests to Dr. S. Samman, at the Human Nutrition Unit, University of Sydney, NSW 2006, Australia.
Received April 18, 1995; accepted September 13, 1995.

Nutritional Biochemistry 7:66–76, 1996

655 Avenue of the Americas, New York, NY 10010

0955-2863/96/$15.00
SSDI 0955-2863(95)00168-9

radical scavenging properties of phenolic compounds, present in red wine, may partly explain the anomaly observed in the coronary heart disease (CHD) rate between the French population who consume wine regularly and have rates of CHD lower than other populations despite similar fat intakes.[26–28] The aims of this review are to evaluate the chemistry of flavonoids, their absorption, metabolism, dietary sources, and association with CHD.

Chemistry of flavonoids

Generic structure and major classifications

Flavonoids are low molecular weight polyphenolic substances based on the flavan nucleus.[29] *Figure 1* shows the generic structure of flavonoids and the numbering system used to distinguish the carbon positions around the molecule. The three phenolic rings are referred to as the A, B, and C (or pyrane) rings. The biochemical activities of flavonoids and their metabolites depend on their chemical structure and the relative orientation of various moieties on the molecule.[30] Flavonoids are classified according to their chemical structure. The major flavonoid classes include flavonols, flavones, flavanones, catechins (or flavanols), anthocyanidins, isoflavones, dihydroflavonols, and chalcones.[16,31–33]

Substitution

Tables 1–3 and *Figure 2* show the major flavonoid classes and some structural variations that have been identified. The structure of flavonoids varies widely within the major classifications, and substitutions include hydrogenation, hydroxylation, methylation, malonylation, sulphation, and glycosylation.[31,32] Many flavonoids occur naturally as flavonoid glycosides,[33,34] and carbohydrate substitutions include D-glucose, L-rhamnose, glucorhamnose, galactose, lignin, and arabinose.[34] Quercitrin, rutin, and robinin are the most common flavonoid glycosides in the diet. They are hydrolyzed by intestinal flora to produce the biologically active aglycone (sugar-free flavonoid).[33] Quercetin is the subject of many studies investigating the biological effects of flavonoids, because it is the predominant flavonoid found in foods.[35]

Polymerization

Flavonoids may be monomeric, dimeric, or oligomeric. Monomers vary greatly in size; for example flavone has a molecular weight of 222 whereas blue anthocyanin has a molecular weight of 1,759.[32] Polymeric compounds, called tannins, are divided into two groups based on their structure: condensed and hydrolyzable.[36] Condensed tannins are polymers of flavonoids[1] and hydrolyzable tannins contain gallic acid (*Figure 3*), or similar compounds, esterified to a carbohydrate.[36] Galloyl groups have iron chelating properties in vitro and are believed to interfere with iron absorption in vivo.[36]

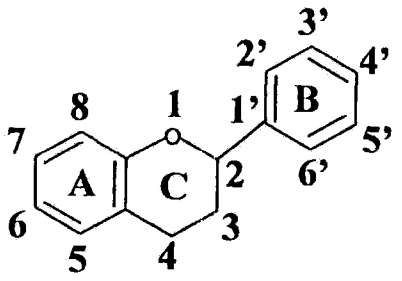


Figure 1 The generic structure of flavonoids.

Tea tannins consist of four main catechin components: epicatechin, epigallocatechin, epicatechin gallate, and epigallocatechin gallate.[37,38] In tea epigallocatechin gallate is the predominant catechin accounting for more than half of the total catechin content.[29,38] Enzymatic oxidation of tea catechins during fermentation of macerated tea leaves produces the dimeric theaflavins and the polymeric thearubigins of black "Indian" tea[29] which produce the brightness and astringency, respectively.[29,33,37] Thearubigins range widely in size between oligomers of four or five flavonoid units to molecules of up to 100 flavonoid units.[29] In contrast to black tea, the flavonoids in green "Chinese" tea occur mostly as monomers because green tea is not fermented during processing.[37] In red wine, tannins are formed by the polymerization of anthocyanins and other flavonoids producing the wine's characteristic colors, flavors, and astringency.[39]

Distribution and function in plants

Over 4,000 types of flavonoid compounds have been identified in vascular plants and these vary in type and quantity due to variations in plant growth, conditions, and maturity.[37] Only a small number of plant species have been examined systematically for their flavonoid content[32] and therefore the identification and quantification of all the types of flavonoids consumed by humans is incomplete.[37] Plants have evolved to produce flavonoids to protect against fungal parasites,[32] herbivores, pathogens, and oxidative cell injury.[39] Conversely, flavonoids produce stimuli to assist in pollination[31] and guide insects to their food source.[32] For example, anthocyanins produce the pink, red, mauve, violet, and blue colors of flowers, fruits, and vegetables.[29]

Absorption and metabolism

Studies in animals

Bravo et al.[40] studied the degradability of the polyphenolic compounds, catechin, and tannic acid in the intestinal tract of rats. Male Wistar rats were fed diets containing catechin or tannic acid (equivalent to 0.5 g/day, assuming a rat weighing 200 g consumes 25 g diet/day), over a 3 week period. There was little fermentation by the gut flora, and less than 5% of the ingested catechin and tannic acid were excreted unchanged in the feces suggesting that absorption of the polyphenolic compounds had occurred.

Although there were no interactions between phenolic compounds and protein digestion,[40] alterations to lipid metabolism have been reported in rats fed diets containing tannic acid and catechin.[38,40] It is postulated that catechin

Table 1 Structure of flavonoids

Flavonoid	Total No. of OH groups	Position of OH groups	Substitutions on the generic structure	Position of the substitutions
Myricetin	6	3,5,7,3′,4′,5′.		
Gossypetin	6	3,5,7,8,3′,4′.		
Quercetagen	6	3,5,6,7,3′,4′.		
Quercetin	5	3,5,7,3′,4′.		
Morin	5	3,5,7,2′,4′.		
Robinetin	5	3,7,3′,4′,5′.		
Myricetrin	5	5,7,3′,4′,5′.	O-Rh	3
Rutin	4	5,7,3′,4′.	O-Ru	3
Kaempferol	4	3,5,7,4′.		
Quercetrin	4	5,7,3′,4′.	O-Rh	3
Fisetin	4	3,7,3′,4′.		
Datiscetin	4	3,5,7,2′.		
Rhamnetin	4	3,5,3′,4′.	O-Me	7
Tamarixetin	4	3,5,7,3′	O-Me	4′
Silybin	3	3,5,7.	O-Lig-O	4′
Galangin	3	3,5,7.		
Kaempferide	3	3,5,7.	O-Me	4′
Diosmin	2	3,3′.	O-Ru, O-Me	5,4′
Robinin	2	5,4′.	O-Gal-Rh, Rh	3,7′
Troxerutin	1	5	O-Ru, O-He, O-He, O-He	3,7,3′,4′.
3-OH-Flavone	1	3		

Rh = rhamnose = 6-deoxy-L-mannose ($C_6H_{12}O_5$); Lig = lignin; Ru = rutinose = 6-O-D-glucose ($C_{12}H_{12}O_{10}$); He = hydroxyethyl (CH_2CH_2OH); Me = methyl (CH_3); Gal = galactose ($C_6H_{12}O_6$).

influences lipid metabolism by increasing bile acid excretion leading to a hypocholesterolemic effect.[38]

Studies in humans

Despite the potentially significant effects of flavonoids on coronary heart disease,[41] information about the absorption, metabolism, and excretion of individual flavonoids in humans is scarce. Some studies report that flavonoids are absorbed after oral administration,[42] although others conclude that they are poorly absorbed and do not reach the general circulation unchanged at measurable concentrations.[43] However, most studies of flavonoid metabolism in humans have examined the metabolism of individual flavonoids taken at pharmacological doses rather than at estimated levels of dietary intake[42,43] of approximately 23[35] to 170 mg/day.[33] Therefore, extrapolation of the results of these studies may be inappropriate to explain the absorption and metabolism of dietary flavonoids.

Das[42] studied the absorption and metabolism of (+)-catechin in six healthy male volunteers following the administration of a single dose of (+)-catechin (92.3 mg/kg of body weight, mean 4.2 g). Within 6 hr, phenols were detected in plasma and returned to baseline by 96 hr. The phenolic compounds were excreted in urine in both free and conjugated forms and included sulphate conjugates. In the feces, approximately 19% of the administered dose was excreted unchanged. There were no adverse side effects reported following the large single oral dose of catechin.[42]

Gugler et al.[43] investigated the metabolism of quercetin in six volunteers (four male and two female) aged between 21 and 32 years. After the oral administration of a single dose of 4 g, no measurable concentrations of the flavonoid or its derivatives were detected in plasma or urine. However, approximately 53% of the oral dose was recovered unchanged in the feces, and it was concluded that 1% of the original 4 g dose of quercetin or approximately 40 mg was absorbed.[43] The estimated average intake of all flavonoids from dietary sources is between 23[35] and 170 mg/day.[33] Therefore, absorption of 40 mg is not discountable. However, studying the metabolism of one flavonoid, namely quercetin, at a single pharmacological dose that greatly exceeds the estimated dietary consumption of flavonoids[33,35] may not explain the metabolism of flavonoids from dietary sources. Humans are unlikely to consume dietary flavonoids individually due to the diversity and wide distribution of flavonoids in foods. Therefore, the results of the study by Gugler et al.[43] may not reflect the mechanisms of absorption and metabolism of dietary flavonoids.

Lipid peroxidation

Polyunsaturated fatty acids (PUFA) present in cell membranes are oxidized by both enzymatic and auto-oxidative peroxidation and by free radical chain reactions.[13] An overabundance of free radicals can lead to uncontrolled chain reactions and LPO[44] resulting in pathological conditions that may include atherosclerosis and cancer.[22] LPO proceeds in three stages: initiation, propagation, and termination.[13,45]

In the initiation stage of LPO, free radicals abstract hydrogen from PUFA to form the lipid radical. In the propagation stage, the lipid radical reacts with molecular oxygen to form the lipid peroxy radical which breaks down to generate more free radicals thus maintaining the chain of reactions. In the termination stage, the free radical species react

Table 2 Structure of flavones

Flavone	Total no. of OH groups	Position of OH groups	Substitutions on the generic structure	Position of the substitutions
Hypolactin	5	5,7,8,3′,4′		
Luteolin	4	5,7,3′,4′		
Scutellarein	4	5,6,7,4′		
Isoorientin	4	5,7,3′,4′	Gluc	6
Orientin	4	5,7,3′,4′	Gluc	8
Apigenin	3	5,7,4′		
Silymarin	3	4,6,3′		
Diosmetin	3	5,7,3′	O-Me	4′
Luteolin-7-glucoside	3	5,3′,4′.	O-Gluc	7
Baicalein	3	5,6,7		
Cirsiliol	3	5,3′,4′	O-Me	7
Sideritoflavone	3	5,3′,4′	O-Me	6,7,8
Pedalitin	3	5,3′,4′	O-Me	7
Vitexin	3	5,7,4′	Gluc	8
Vicenin-2	3	5,7,4′	Gluc	6,8
Pinocembrin	2	5,7		
Hispidulin	2	5,7	O-Me	4′
5,7-Dihydroxytrimethoxy- flavone	2	5,7	O-Me	3,4′,5′
Gardenin-D	2	5,3′	O-Me	6,7,8,4′
Acetetin	2	5,7	O-Me	4′
Chrysin	2	5,7		
Cirsimaritin	2	5,4′	O-Me	6,7
Xanthomicrol	2	5,4′	O-Me	6,7,8
8-Methoxycirisilincol	2	5,4′	O-Me	6,7,8,3′
5-O-Demethylnobiletin	1	5	O-Me	6,7,8,3′,4′
Techtochyrsin	1	5	O-Me	7
Flavone	0			

Me = methyl = (CH_3); Gluc = glucose.

together or with antioxidants to form inert products.[13,29,45] LPO can be suppressed by enzymatic inactivation of free radicals[22,34] and antioxidants that inhibit the initiation stage and/or accelerate the termination stage.[45] Thus, LPO can be prevented at the initiation stage by free radical scavengers and singlet oxygen quenchers, and the propagation chain reaction can be broken by peroxy-radical scavengers.[13]

The antioxidant and chelating properties of flavonoids

Flavonoids inhibit LPO in vitro at the initiation stage by acting as scavengers of superoxide anions and hydroxyl radicals.[13,20] It has been proposed that flavonoids terminate chain radical reactions by donating hydrogen atoms to the peroxy radical forming a flavonoid radical.[13,20] The flavonoid radical in turn reacts with free radicals thus terminating the propagating chain.[13,46] In addition to their antioxidative properties, some flavonoids act as metal-chelating agents and inhibit the superoxide-driven Fenton reaction, which is an important source of active oxygen radicals.[20] However, there is no clear evidence of the antioxidant and free radical scavenging effects of flavonoids in vivo.[40]

Structure-activity relationships of lipid peroxidation inhibition by flavonoids

The inhibition of LPO is influenced by a number of structural features of flavonoids:

(1) The presence of a hydroxyl group in position three (3-OH) of the C ring.[2,7,20,22,47,48] The flavonoid aglycones that have a 3-OH group such as fisetin, (+)-catechin, quercetin, myricetin, and morin are potent inhibitors of LPO compared with those that lack a 3-OH substitution such as diosmetin, apigenin (flavones), hesperetin, and naringenin (flavanones).[47]

(2) A double bond between carbons two and three (C2-C3) of the C ring.[2,19,48,49] Hydrogenation of this bond decreases the antiperoxidative effects.[22,49]

(3) The carbonyl group at C-4 of the C ring is necessary for antiperoxidant activity in some studies[2,19,48] but not others.[7,49] Catechin lacks a C-4 carbonyl and has lower hydroxyl radical scavenging potency than quercetrin which has a C-4 carbonyl group.[50]

(4) The number of hydroxyl groups.[2,7,19,49] The importance of polyhydroxylated substitutes on the A and B rings was demonstrated by comparing quercetin, quercetin, myricetin, myricetrin, phloretin, (+)-catechin, morin, and fisetin with apigenin, hesperetin, hesperidin, naringenin, naringin, chrysin, and 3-hydroxyflavone.[2] In the former group, each of the flavonoids has between four and six hydroxyl substitutions while the latter group has between one and three hydroxyl groups. The hydroxyl radical scavenging activity of flavonoids increases with the number of hydroxyl groups substituted on the B ring, especially at C-3′, and decreases rapidly as the number of hydroxyl groups decreases.[2] Myricetin (hydroxylation pattern: 3,5,7,3′,4′,5′) has greater hydroxyl

Table 3 Structure of flavanones, catechins, anthocyanidins, isoflavones, dihydroflavonols, and chalcones

Flavonoid classes	Total no. of OH groups	Position of OH groups	Substituions on the generic structure	Position of the substitutions
Flavanones				
Eriodictyol	4	5,7,3′,4′		
Hesperetin	3	5,7,3′	O-Me	4′
Naringenin	3	5,7,4′		
Hesperidin	2	5,3′	Rh-Gluc, O-Me	7,4′
Naringin	2	7,4′	O-Rh-Gluc	5
Catechins				
Leucocyanidol	6	3,4,5,7,3′,4′		
(+)-Catechin	5	3,5,7,3′,4′		
(+)-Epicatechin	5	3,5,7,3′,4′		
Anthocyanidins				
Delphinidin chloride	6	3,5,7,3′,4′,5′	Chloride	1
Delphinidin	6	3,5,7,3′,4′,5′		
Cyanidin chloride	5	3,5,7,3′,4′	Chloride	1
Cyanidin	5	3,5,7,3′,4′		
Petunidin	5	3,5,7,4′,5′	O-Me	3′
Peonidin	4	3,5,7,4′	O-Me	3′
Malvidin	4	3,5,7,4	O-Me	3′,5′
Isoflavones				
Genistein	3	5,7,4′		
Diadzein	2	7,4′		
Dihydroflavonols				
Taxifolin	5	3,5,7,3′,4′		
Fustin	4	3,7,3′,4′		
Chalcones				
Butein	4	3,4,4′,6		
Phloretin	4	4,2′,4′,6′		
Phloridzin	3	4′,2′,4	O-Gluc	6

Gluc = glucose; Me = methyl (CH_3); Rh = rhamnose = 6-deoxy-L-mannose ($C_6H_{12}O_5$).

radical scavenging activity than kaempferol (hydroxylation pattern: 3,5,7,4′).[50]

(5) The pattern of hydroxylation.[49] Hydroxyl groups on positions C-5 and C-7 of the A ring[7,41] C-3′ and C-4′ of the B ring[7,21,51]; and position C-3 of the C ring[49] appear to contribute to the inhibition of LPO. Flavonols require a C-2′ hydroxyl and the pyrogallol group (C-3′, C-4′, C-5′) for antiperoxidative activity.[49]

(6) The presence of a sugar moiety.[2,18] Flavonoid aglycones such as apigenin, naringenin, hesperetin, diosmetin, quercetin, phloretin and myricetin are more effective in inhibiting malondialdehyde (MDA) production than their corresponding glycosides.[2,18,19] The sugar moiety reduces the antiperoxidation efficiency of adjacent hydroxyl groups due to steric hindrance.[2,7,22,49] However, flavonoid glycosides, such as quercetin and rutin, are hydrolyzed to their corresponding aglycones by human intestinal flora.[3,51] Therefore, the findings that flavonoid glycosides have lower antiperoxidative potency than aglycone flavonoids in vitro may not be relevant to the in vivo effects of flavonoids.

(7) Methoxyl groups reduce antiperoxidative efficiency of flavonoids in vitro due to steric hindrance.[49]

(8) Flavonoids having both a C-4 carbonyl group and a C-3 or C-5 hydroxyl group, such as rutin and quercetin, form chelates with iron ions.[21,39,49] The ability of flavonoids to sequester metal ions may contribute to their antiperoxidative properties by preventing the formation of free radicals in the Fenton system.[20,34,39,48] Moreover, flavonoids retain their free radical scavenging activities after forming complexes with iron ions.[20] Thus the formation of metal ion chelates is one antioxidant mechanism of flavonoids.[20–22]

Flavonoids and coronary heart disease

Role of oxidized LDL in atherogenesis

Elevated plasma LDL cholesterol concentrations are associated with accelerated atherosclerosis.[24] Atheromatous lesions develop in the subendothelial space due to the accumulation of cholesteryl esters in macrophages forming foam cells. Until recently, the mechanism of foam cell formation was unclear because macrophages have few LDL receptors, and paradoxically these receptors are down-regulated as plasma LDL concentrations increase.[52] Goldstein et al.[53] were the first to demonstrate that a chemically modified (acetylated) LDL in vitro is recognized by specific receptors (scavenger receptors) on the macrophage. Consequently, modified LDL is endocytosed at a much higher rate than native LDL. Scavenger receptors that recognize oxidatively modified LDL have since been identified[25,54,55] and there is much evidence that oxidized LDL is responsible for cholesterol loading of macrophages, foam cell formation, and atherogenesis.

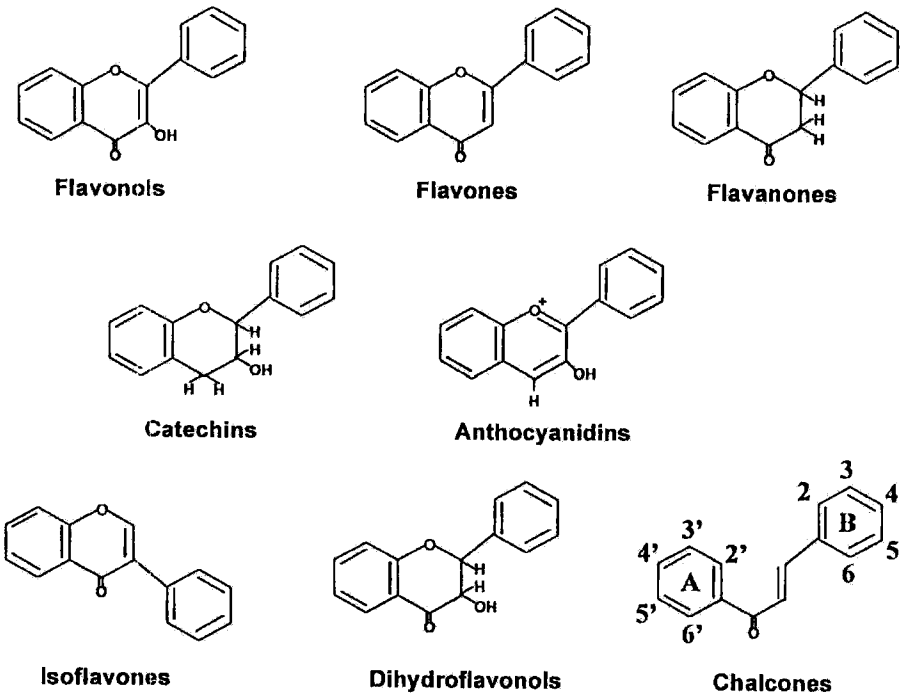
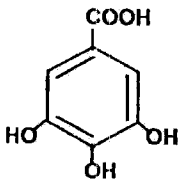


Figure 2 The structures of the major classes of flavonoids.

LDL is oxidized by free radicals generated from endothelial cells, monocyte-derived macrophages, and smooth muscle cells, resulting in several chemical and physical changes of LDL.[24,56,57] Oxidized LDL is chemotactic for macrophages promoting their residence in the intima, cytotoxic to the endothelium, chemoattractant for monocytes, and rapidly accumulated by resident macrophages.[24,57,58] Therefore, it has been hypothesized that oxidized LDL initiates and promotes atherogenesis in several ways.

LDL particles contain endogenous antioxidants including α- and γ-tocopherols, β-carotene, lycopine, and retinyl stearate.[41,59–61] LDL oxidation in vitro exhibits a lag phase corresponding to the time required for the endogenous antioxidants in LDL to be consumed.[41,59] Exogenous antioxidants, such as α-tocopherol, butylhydroxytoluene (BHT)[62] urate, ascorbic acid, and probucol, and metal chelators, such as EDTA, can protract the lag phase or even prevent LDL oxidation in vitro.[63–65] Recent reports show an inverse association between dietary intake of phenolic antioxidants, including α-tocopherol, and CHD.[66] It is postulated that the antioxidant effects of dietary α-tocopherol in a similar manner to flavonoids may in part explain the French paradox.[26,67] Hence, a range of minor dietary factors, including flavonoids and α-tocopherol, may collectively act as effective antioxidants in the prevention of CHD.



Gallic Acid

Figure 3 The structure of gallic acid.

Epidemiological evidence for the cardioprotective effects of flavonoids

The Zutphen Elderly Study[68] is the only published epidemiological study that examines the relationship between dietary flavonoid intake and the risk of CHD. The Zutphen study assessed the flavonoid intake of 805 men aged 65 to 84 years. There was a significant inverse association between dietary flavonoid intake and mortality from CHD and an inverse but weaker relation with the incidence of myocardial infarction. These findings were significant after adjusting for known major confounders.[68]

The average flavonoid intake in this population was estimated to be 26 mg/day, and the major contributors to these estimates were tea, 61%, onions, 13%, and apples, 10%.[68] Flavonoid intake and tea consumption were highly correlated, and both were inversely associated with death from CHD.[68] However, the total flavonoid intake had a greater effect on CHD mortality than tea itself, suggesting that flavonoids rather than other substances in tea were responsible for the protection against CHD.

The flavonoid content of many foods commonly eaten in The Netherlands has been analyzed.[69,70] However, more research is required to identify the flavonoid content of foods consumed in other countries. Also, further epidemiological studies are needed to confirm these findings and identify other foods that may, due to their high flavonoid content, have potential cardioprotective properties.

Inhibition of LDL oxidation in vitro by flavonoids

A number of aglycone flavonoids are potent inhibitors of oxidative modification of LDL in vitro by macrophages or copper ions.[41] Phenolic compounds isolated from red wine inhibit the copper catalyzed oxidation of LDL in vitro significantly more than α-tocopherol.[26] However, the ability of flavonoids to protect LDL from oxidative modification in vivo depends on their absorption, metabolism, and in particular the association of flavonoids with lipoproteins. Recent studies in humans suggest that polyphenols obtained through drinking red wine associate with plasma LDL[71] and are significantly more effective than white wine in reducing the oxidizability of whole plasma[71,72] and of plasma LDL.[71]

The exact mechanisms by which flavonoids inhibit LDL oxidation are uncertain. Flavonoids may reduce the formation of free radicals[2,20,21,41,46,48–50] or protect the α-tocopherol in LDL from oxidation by being oxidized themselves in preference to α-tocopherol, thus delaying the start of LPO.[41] Alternatively, flavonoids may regenerate α-tocopherol by donating a hydrogen atom to the α-tocopherol radical.[13,26] Also, flavonoids may inhibit LDL oxidation by chelating divalent metal ions and thus reducing the formation of free radicals induced by Fenton reactions.[20,39,41,49]

There have been insufficient tests of the protective effects of flavonoids against LDL oxidation to make definitive statements about their structure-activity relationships. However, hydroxylation of the flavone nucleus appears to be advantageous because flavone itself is a poor inhibitor of LDL oxidation, whereas polyhydroxylated aglycone flavonoids such as quercetin, morin, hypoleatin, fisetin, gossypetin, and galangin are potent inhibitors of LDL oxidation.[41] These findings are consistent with previous studies of the structure-activity relationships of flavonoids in the inhibition of LPO.[2,7,18,19,21,49]

The ability of (+)-catechin to inhibit LDL oxidation induced by copper and several cell lines including mouse macrophages, human monocyte-derived macrophages, and vascular endothelial cells isolated from human umbilical cords have been investigated.[57] As expected, LDL modified by cells or copper-induced oxidation was endocytosed and degraded by human macrophages more quickly than native LDL. However, in the presence of (+)-catechin, the rate of endocytosis and degradation by macrophages was similar to that of native LDL.[57] This provides further evidence that flavonoids may protect LDL from oxidative modification and therefore protect against atherosclerosis if they are delivered to the subendothelial space where LDL oxidation occurs.

In addition to the inhibition of LDL oxidation, flavonoids such as catechin, rutin, and quercetin strongly inhibit LPO and the subsequent cytotoxicity of oxidized LDL.[63,65] Moreover, cells preincubated with these flavonoids were resistant to the cytotoxic effects of previously oxidized LDL.[63,65] The postulated mechanisms by which flavonoids guard against cytotoxicity of oxidized LDL are consistent with the antioxidant and free radical scavenging properties.[2,20,21,41,46,48–50]

Antithrombotic and vasoprotective effects of flavonoids

Platelet-blood vessel interactions are implicated in the development of thrombosis and atherosclerosis. Particular flavonoids inhibit platelet aggregation and adhesion thus reducing thrombotic tendencies.[8,10–12,15,22,73,74] However, the antiaggregatory effects of flavonoids cannot be attributed to a single biochemical mechanism because they appear to influence several pathways involved in platelet function[12,75,76] such as the inhibition of the enzymes cyclo-oxygenase and lipoxygenase involved in arachidonic acid metabolism in platelets. Also flavonoids inhibit platelet aggregation by antagonizing thromboxane formation and thromboxane receptor function.[12] One of the most potent mechanisms by which flavonoids appear to inhibit platelet aggregation is by mediating increases in platelet cyclic AMP (cAMP) levels by either stimulation of adenylate cyclase or inhibition of cAMP phosphodiesterase (PDE) activity.[6,9–11,73,76–78]

The antioxidant actions of flavonoids appear to participate in their antithrombotic action.[8,15,73] The antithrombotic and vasoprotective actions of quercetin, rutin, and other flavonoids have been attributed to their ability to bind to platelet membranes and scavenge free radicals.[8] By their antioxidant actions, flavonoids restore the biosynthesis and action of endothelial prostacyclin and endothelial derived relaxing factor (EDRF) both of which are inhibited by free radicals.[8,10,46] However, the lack of antioxidant actions of sideritoflavone and cirsiliol, which are potent LPO inhibitors, suggests that some flavonoids may inhibit arachidonic acid metabolism and platelet function by flavonoid-enzyme interactions rather than by antioxidant effects.[22]

The structural features required for flavonoids to inhibit human platelet aggregation and adhesion are similar to those associated with the antioxidant function of flavonoids and the inhibition of cAMP PDE and include a double bond between C-2 and C-3, a 3-OH group, and a carbonyl group at C-4.[73] The inhibitory effect of flavonoids on platelet function is diminished by glycosylation at C-3,[15] saturation of the double bond between C-2 and C-3, and polyhydroxylation.[73] Thus flavonoid glycosides and flavanone derivatives do not appear to affect platelet function.

Regular consumption of red wine is linked to decreased platelet aggregation and the prevention of CHD.[27] However, withdrawal from beer or spirits for at

least 12 hr by people who regularly consume these beverages is associated with rebound platelet reactivity and an increased risk of thrombosis.[79] In rats withdrawal from red wine resulted in a 59% decrease in rebound platelet reactivity compared with increases following withdrawal from ethanol or white wine.[79] It is postulated that the antioxidant properties of phenolic compounds in red wine reduce platelet aggregation and inhibit LPO in vitro.[26,79] If reproduced in humans, the protective effects of red wine against platelet aggregation may partly explain the long-term advantages of consuming moderate amounts of red wine over other alcoholic beverages.[80]

In addition to their antiaggregatory effects, flavonoids appear to increase vasodilation by inducing vascular smooth muscle relaxation which may be mediated by the inhibition of protein kinase C, PDEs, or by decreased cellular uptake of calcium.[6]

Dietary intake and food sources of flavonoids

Until recently, data on human flavonoid intake were obtained from Kühnau[33] who estimated the average intake of all dietary flavonoids in the USA to be approximately 1 g/day (expressed as glycosides) of which about 170 mg (expressed as aglycones) consisted of flavonols, flavanones, and flavones. These values have been widely quoted[1,2,4,51]; however, they are based on food analysis techniques now considered inappropriate.[81] Furthermore, estimates of flavonoid intake were based on analysis of whole foods and estimates of the average American diet extrapolated from the Organization for Economic Cooperation and Development (OECD) food consumption statistics[81] thus overestimating food intake and consequently the average flavonoid intake.

The content of the flavonols quercetin, kaempferol, and myricetin and the flavones luteolin and apigenin in 28 vegetables, 9 fruits, and beverages commonly consumed in The Netherlands was analyzed using more recent and advanced methodologies.[35,69,70] Based on these analyses and using data from the Dutch National Food Consumption Survey 1987–88, the average dietary flavonoid intake in The Netherlands was estimated to be approximately 23 mg/day (expressed as aglycones).[35]

Quercetin was the major dietary flavonoid (mean intake 16 mg/day), followed by kaempferol (4 mg/day), myricetin (1.4 mg/day), luteolin (0.92 mg/day), and apigenin (0.69 mg/day). The greatest dietary sources of flavonoids were: tea, 48% of total intake; onions, 29%; and apples, 7%. The average consumption was: tea, 2 cups/day (294 ± 310 mL); onions, 16 ± 32 g/day; and apples, 45 ± 71 g/day. Thus, these levels of flavonoid intake were achieved without unusually high consumption of these foods. Red wine is also a rich sources of flavonoids and contains approximately 22.5 mg/L (3.8 mg/170 mL glass).[69]

The estimated flavonoid intake of 23 mg/day was based on the content of five flavonoids in Dutch foods; therefore, the total flavonoid intake in this population may be higher. Moreover, this estimation is based on analysis of foods commonly consumed in The Netherlands and thus may not represent the flavonoid content of foods consumed in other countries. A systematic analysis of the flavonoid content of foods consumed in other countries is required to estimate flavonoid intakes in other populations.

Therapeutic potential of flavonoids

Concentrated forms of flavonoids, such as propolis (a resinous substance obtained by bees from plants for use as glue in their hives) has been used for centuries to treat a wide variety of human conditions including inflammation, allergy, headache, cancer, viral infections, the common cold, bee stings, and gastric and duodenal ulcers.[34] Flavonoid preparations have been used widely in medical practice for over 40 years to treat disorders of peripheral circulation.[82] Over 100 preparations containing flavonoids, including cianidanol, diosmetin, hesperidin, leucocianidin, rutin, and troxerutin, are marketed in France and Switzerland.[82] Many of the alleged effects of pharmacological doses of flavonoids have been linked to their known functions as strong antioxidants (including vitamin C–sparing properties), free radical scavengers, metal chelators, and enzyme-flavonoid interactions. However, therapeutic preparations of flavonoids have yet to pass controlled clinical trials.

Red wine is a rich source of flavonoids, and regular red wine consumption is associated with a decreased risk of CHD and may partly explain the French paradox. However, an indirect adverse effect of encouraging the consumption of red wine[83] is the potential to increase the risk of cirrhosis associated with alcohol consumption. While the risk/benefit ratio may vary for individuals, the use of alcohol for cardioprotective purposes should not be encouraged as a public health measure.

Most research conducted on the biochemical effects of flavonoids has focused on the potential of flavonoids as pharmaceuticals[18,20,34] rather than the possible health benefits of obtaining flavonoids in the diet. The reported benefits of flavonoids have mostly been inferred from results at pharmacological concentrations. Consequently, the reported effects of pharmacological doses of flavonoids are primarily of pharmacological rather than dietary significance. Thus more research is needed to elucidate the biochemical effects of flavonoids in the diet.

Possible adverse effects of flavonoids

Adverse reactions from flavonoids have been reported following administration of chronic pharmacological doses[82] that exceed the estimated dietary intake of 23[35] to 170 mg/day.[33] Toxic effects that have been documented from doses of 1 to 1.5 g/day of flavonoid drugs such as cianidanol include acute renal failure, hemolytic anemia, thrombocytopenia, hepatitis, fever, and skin reactions.[80] In one study, quercetin is reported to have induced bladder cancer in rats when consumed at the level of 2% in the diet.[80] However, these results were not confirmed in another study where quercetin was

administered at doses up to 10% of the diet.[84] Importantly, in a diet containing a wide variety of foods flavonoids are unlikely to be consumed in toxic quantities because foods originating from plants contain many diverse types of flavonoids in varying quantities.

Tea is a rich source of flavonoids[69] but black tea due to the presence of hydrolyzable tannins (tannic acid) is a well known inhibitor of iron absorption. Phenolic compounds, such as phenolic monomers, polyphenols, and tannins are considered to interfere with iron absorption by forming insoluble complexes in the gastrointestinal lumen thus reducing iron bioavailability.[36] Phenolic molecules with aromatic rings bearing two hydroxyls (catechol group) or three hydroxyls (galloyl group) on adjacent carbons have iron binding properties in vitro. However, the inhibition of iron absorption by phenolic compounds in vivo has been positively correlated with the presence of galloyl groups but not catechol groups.[36] Research is needed to elucidate the relationship between iron absorption and the chelation of iron by the C-4 carbonyl group of flavonols, flavones, and flavanones.

Conclusion

Epidemiological and in vitro evidence of antioxidant and cardioprotective effects support the hypothesis that flavonoids benefit health. The inhibition of LDL oxidation and platelet aggregation by flavonoids suggests that regular consumption of foods containing flavonoids and moderate consumption of red wine may protect against atherosclerosis and thrombotic tendency. The large contribution of flavonoids to the diet from tea, onions, and apples suggests that these foods may have greater nutritional benefits than previously recognized as they appear to constitute a major source of dietary antioxidants. More research is required for further elucidation of the mechanisms of flavonoid absorption, metabolism, and biochemical action and interaction with other nutrients in vivo. Furthermore, research is needed to identify the mechanisms by which flavonoids contribute to the amelioration of atherosclerosis and reduce the risk of morbidity and mortality from CHD.

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